

appreciate that the protein fragments or peptides may be separated by a spacer molecule such as, for example, a peptide, consisting of one or more amino acids. Generally, the spacer will have no specific biological activity other than to join the desired protein fragments or peptides together, or to preserve some minimum distance or other spatial relationship between them. However, the constituent amino acids of the spacer may be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity. Nucleotide sequences encoding for the production of residues which may be useful in purification of the expressed recombinant protein may be built into the vector. Such sequences are known in the art. For example, a nucleotide sequence encoding for a poly histidine sequence may be added to a vector to facilitate purification of the expressed recombinant protein on a nickel column.

Once expressed, recombinant peptides, polypeptides and proteins can be purified according to standard procedures known to one of ordinary skill in the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Substantially pure compositions of about 50 to 99% homogeneity are preferred, and 80 to 95% or greater homogeneity are most preferred for use as therapeutic agents.

Also, molecules capable of forming some of the named proteins can be mixed with other polymers during electroprocessing to obtain desired properties for uses of the formed protein in the matrix.

Throughout this application the term "solution" is used to describe the liquid in the reservoirs of the electroprocessing method. The term is defined broadly to include any liquids that contain materials to be electroprocessed. It is to be understood that any solutions capable of forming a material during electroprocessing are included within the scope of the present invention. In this application, the term "solution" also refers to suspensions or emulsions containing the material or anything to be electrodeposited. "Solutions" can be in organic or biologically compatible forms. This broad definition is appropriate in view of the large number of solvents or other liquids and carrier molecules, such as polyethylene glycol (PEG), that can be used in the many variations of electroprocessing. In this application, the term "solution" also refers to melts, hydrated gels and suspensions containing the materials, substances or anything to be electrodeposited.

### *Solvents*

Any solvent that will allow delivery of the material or substance to the orifice or tip of a syringe under such conditions that the material or substance will be processed as desired may be used for dissolving or suspending the material or the substance to be electroprocessed. Solvents useful for dissolving or suspending a material or a substance will depend on the material or substance. Electrospinning techniques often require more specific solvent conditions. For example, non cross-linked fibrin monomer can be electrodeposited or electrospun from solvents such as urea, monochloroacetic acid, water, 2,2,2-trifluoroethanol, or 1,1,1,3,3,3-hexafluoro-2-propanol (also known as hexafluoroisopropanol or HFIP). Collagen can be electrodeposited as a solution or suspension in water, 2,2,2-trifluoroethanol, or HFIP. Elastin can be electrodeposited as a solution or suspension in water, 2,2,2-trifluoroethanol, isopropanol, or HFIP. Other lower order alcohols, especially halogenated alcohols, may be used. Proteins and peptides associated with membranes are often hydrophobic and thus cannot dissolve in aqueous solutions. Such proteins can be dissolved in organic solvents such as methanol, chloroform, and trifluoroethanol (TFE). Any other solvents known to one of skill in the protein chemical art may be used, for example solvents useful in chromatography, especially high performance liquid chromatography. Proteins and peptides are also soluble, for example, in HFIP, hexafluoroacetone, chloroalcohols in conjugation with aqueous solutions of mineral acids, dimethylacetamide containing 5% lithium chloride, in very dilute acids such as acetic acid and formic acid. N-methyl morpholine-N-oxide is another solvent that can be used with many polypeptides.

In functional terms, solvents used for electroprocessing have the principal role of creating a mixture with a polymer, or polymers, such that electroprocessing is feasible. The concentration of a given solvent is often an important consideration in determining the type of electroprocessing that will occur. For example, in electrospraying, the solvent should assist in the dispersion of droplets of electroprocessed material so that the initial jet of liquid disintegrates into droplets. Accordingly, solvents used in electrospraying should not create forces that will stabilize an unconfined liquid column. In electrospinning, interactions between molecules of electroprocessed material stabilize the jet, leading to fiber formation. Accordingly, for electrospun embodiments, the solvent should sufficiently dissolve or disperse the polymer to

prevent the jet from disintegrating into droplets and should thereby allow formation of a stable jet in the form of a fiber. In some embodiments, the transition from electrospraying to electrospinning can be determined by examining Brookfield viscosity measurements for polymer solutions as a function of concentration. Brookfield viscosity increases as concentration of a polymer or other material to be electroprocessed increases. Above a critical concentration associated with extensive chain entanglements of materials, however, the Brookfield viscosity will increase more rapidly with concentration, as opposed to a more gradual, linear rise with concentration at lower concentrations. For example, the Brookfield viscosity of a poly(lactide) sample obtained from Alkermes dissolved in chloroform shows an upturn in the Brookfield viscosity/concentration plot at approximately 7-8 % w/v. A sample of poly(ethylene-co-vinyl acetate) from Dupont (ELVAX 40W) shows an upturn at 14-15 % w/v. In both cases, these departures from linearity approximately coincide with the transition from electrospraying to electrospinning.

#### *Compositions of the present invention*

##### *The electroprocessed material*

One component of the compositions of the present invention is the electroprocessed material. As defined above, the electroprocessed material of the present invention can include natural materials, synthetic materials, or combinations thereof. Examples include but are not limited to amino acids, peptides, denatured peptides such as gelatin from denatured collagen, polypeptides, proteins, carbohydrates, lipids, nucleic acids, glycoproteins, lipoproteins, glycolipids, glycosaminoglycans, and proteoglycans.

Some preferred materials are naturally occurring extracellular matrix materials and blends of naturally occurring extracellular matrix materials, including but not limited to collagen, fibrin, elastin, laminin, fibronectin, hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, heparin sulfate, heparin, and keratan sulfate, and proteoglycans. These materials may be isolated from humans or other animals or cells or synthetically manufactured. Some especially preferred natural matrix materials are collagen and fibrin and fibronectin. Also included are crude extracts of tissue, extracellular matrix material, extracts of non-natural tissue, or extracellular matrix materials (i.e. extracts of cancerous tissue), alone or in combination.